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Research Report

# Ascophyllum nodosum enriched bread reduces subsequent energy intake with no effect on post-prandial glucose and cholesterol in healthy, overweight males. A pilot study $\stackrel{\star}{}$

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# ABSTRACT

It is well recognised that the consumption of seaweed isolates (such as alginate) successfully reduce energy intake and modulate glycaemic and cholesterolaemic responses. However, to date, the effect of adding whole seaweed to bread has not been widely investigated. Hence, this study aims to investigate the acceptability of *Ascophyllum nodosum* enriched bread as part of a meal, and measure its effect on energy intake and nutrient absorption in overweight, healthy males to see if it has a similar impact. Results from the acceptability study, (79 untrained sensory panellists) indicated that it is acceptable to incorporate seaweed (*A. nodosum*) into a staple food such as bread at concentrations of up to 4% per 400 g wholemeal loaf. A single blind cross over trial (n = 12 males, aged 40.1 ± 12.5 years; BMI 30.8 ± 4.4 kg/m<sup>2</sup>) was used to compare energy intake and nutrient uptake after a breakfast meal using the enriched bread (4% *A. nodosum*) against the control bread (0% *A. nodosum*). Consumption of the enriched bread at breatfast led to a significant reduction (16.4%) in energy intake at a test meal 4 h later. Differences between treatment arms for area under the curve, peak values, and time of peak for blood glucose and cholesterol were not significant. Further investigation of potential mechanisms of action is warranted.

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# Introduction

Obesity is described as an excess accumulation of body fat to the detriment of health leading to an increased risk of mortality (Sorensen, Virtue, & Vidal-plug, 2010). Recent UK data suggest that in 2008, 61% of adults were overweight or obese, with 24% classified as obese (NHS Information Centre, 2009). It is evident that the increased prevalence of overweight individuals has been accompanied by a parallel rise in numbers of obese individuals (Foresight, 2007). With an increasing body mass index (BMI), comes an increased risk of the development of type 2 diabetes mellitus, hypertension, general cardiovascular disease, certain cancers (Kopelman, 2007) and poor psychosocial well-being (Dixon, Dixon, & O'Brien, 2003). The direct and indirect costs of treating overweight and obesity in England are extensive and are anticipated to rise in parallel with average BMIs (Foresight, 2007). Obesity is a multifactorial disease (Martinez,

2007) and the aetiological factors involved act both independently and dependently (Haskell et al., 2007). In response to the problem, numerous prophylactic and lifestyle approaches have been developed although the majority appear, in the long term, relatively unsuccessful with only an estimated 20% of individuals deemed "successful" in achieving weight loss (Wing & Phelan, 2005).

As body weight is determined by long term energy balance, manipulating the satiating capacity of food may prove beneficial in the control of food intake, and potentially therefore, weight regulation. The addition of fibre to the diet may be particularly beneficial in this respect (Birketvedt, Aaseth, Florholmen, & Ryttig, 2000; Howarth, Saltzman, & Roberts, 2001; Liu, Willett, Manson, Hu, Rosner, & Colditz, 2003; O'Neil, Zanovec, Cho, & Nicklas, 2010; Slavin, 2005).

For centuries, seaweed (a source of dietary fibre), has been a traditional part of the Asian diet (Jiménez-Escrig & Sánchez-Muniz, 2000) however consumption is comparatively low in the UK (Rose et al., 2007) where typically, the only consumption of seaweed is as isolated hydrocolloids used in the food industry as thickening and stabilising ingredients (Brownlee et al., 2005). However, it is becoming increasingly well recognised for its nutritional properties. Notably, seaweed contains favourable amounts of a variety of polysaccharides, dietary fibre, minerals (iodine and calcium) and polyphenols (Burtin, 2003; MacArtain, Gill, Brooks, Campbell, & Rowland, 2007). Seaweed isolates (for example alginates) used

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in appetite research have predominantly yielded encouraging results by decreasing free-living energy intake (Paxman, Richardson, Dettmar, & Corfe, 2008a), reducing cholesterol absorption in rats (Kimura, Watanabe, & Okuda, 1996; Seal & Mathers, 2001) and post-prandial, BMI dependent cholesterolaemia in humans (Paxman, Richardson, Dettmar, & Corfe, 2008b), reducing peak glucose (Williams et al., 2004) and glycaemic response (Wolf et al., 2002), increasing feelings of fullness and decreasing feelings of hunger (Hoad et al., 2004). However, not all studies have shown this modulation of appetite markers. Mattes (2007) found that daily consumption of an alginate enriched breakfast bar had no effect on appetite ratings or energy intake over a 5 day period.

Whilst there is growing evidence to suggest the use of seaweed isolates may be beneficial to health, there appears to be a paucity of evidence surrounding the use of whole seaweed as an ingredient. As consumption of seaweed remains highest in Asian populations most observational studies investigating seaweed ingestion have been conducted in this region, where is has been shown longitudinally to reduce the risk of breast cancer (Yang et al., 2001), osteoporosis (Nakayama, Sakauchi, & Mori, 2008), cardiovascular mortality (Shimazu et al., 2007) as well as type 2 diabetes and prediabetes (Lee, Kim, Vitek, & Nam, 2010). Wholefoods (such as seaweeds) are also an attractive option to food manufacturers and consumers in the light of clean declaration initiatives which are now popular in the food industry. To date, no appetite research has been conducted using seaweed as a whole food ingredient. However, as the prevalence of overweight and obesity are rife in the UK it seems appropriate to investigate its appetite modulating potential.

The aim of this study was to assess the acceptability of seaweed-enriched bread as part of a breakfast meal, and to determine its effects on human energy intake, appetite sensations, and postprandial glycaemia and cholesterolaemia.

#### Methods

The study took place in two stages: an acceptability study followed by a satiety study. In each phase participants gave full informed written consent and procedures for both phases were approved by the appropriate local ethics committee (reference number CFI/2009/RE06).

# Study 1: Acceptability study

As palatability can modulate food intake (Robinson, Gray, Yeomans, & French, 2005; Yeomans, Chambers, Blumenthal, & Blake, 2008), it is important to evaluate the sensory acceptability of test foods (Mattes, Hollis, Hayes, & Stunkard, 2005). In this paper, the terms palatability and sensory acceptability have been used interchangeably similar to some previous studies (Archer, Johnson, Devereux, & Baxter, 2004; Killinger, Calkins, Umberger, Feuz, & Eskridge, 2004; Pelletier & Dhanaraj, 2006). Seventy-nine untrained sensory panellists aged between 18 and 65 years (40 males, 39 females) were recruited to assess the sensory acceptability of 5 samples of wholemeal bread containing 0% (control), 1%, 2%, 3%, and 4% Ascophyllum nodosum (Seagreens<sup>®</sup> Ltd., West Sussex, UK) per 400 g loaf (Table 1) as part of a breakfast meal.

In the acceptability study, bread samples were toasted on each side for 1 min, cut with a pastry cutter (7.5 cm diameter) to remove crusts and topped with scrambled eggs (ingredients described by McCance and Widdowson in The Composition of Food, Food Standards Agency & Institute of Food Research, 2002). Slice depth was kept consistent using an industrial slicer to ensure samples looked similar – an important factor in the sensory study. Participants did not need to finish each sample. Samples were randomly coded using 3 digit blinding codes and were presented in a random order.

In accordance with standard protocol (Mailgaard, Civille, & Carr, 2006), five sensory attributes (appearance, aroma, taste, texture, aftertaste), as well as overall acceptability were evaluated on touch screen operated visual analogue scales with extremes varying from extremely unacceptable (1) to extremely acceptable (9) using industry standard FIZZ software (Version 2.10c, Biosystemes, France). A score of 5 was used as a cut off for lower level acceptability (Mexis, Badeka, Riganakos, & Kontominos, 2010). A timed break of 1 min was enforced between samples, during which panellists consumed water (<200 mL, Brontë Natural Spring water Ltd. (UK)) and crackers (Carr's Water Biscuit, United Biscuits (UK) Ltd.) to cleanse their palates. Tests were conducted silently in temperature controlled (22–24 °C) individual booths, with standardised 'natural' lighting, and positive-air flow. Results were analysed using one-way repeated measures ANOVA and Bonferroni post hoc analyses on SPSS V17.0 (SPSS Inc., Chicago, USA).

#### Study 2: Satiety study

Twelve males, aged between 18 and 65 years (mean age 40.1 ± 12.5 years) self reported as overweight (BMI  $\ge 25 \text{ kg/m}^2$ ) but otherwise healthy were recruited to take part in this study. Consistent with other research in the area of dietary fibre and appetite (Paxman et al., 2008a), the following exclusion criteria were applied to the study: individuals suffering from irritable bowel syndrome, inflammatory bowel disease, Cushing's syndrome, dumping syndrome, severe constipation, severe diarrhoea or coeliac disease, type 1 diabetes, food allergies or any serious medical condition. The study had a single blind, cross-over design. A wash out period of 1 week was considered appropriate in order to eliminate potential carry over effects.

Recruitment took place via email, online newsletters, and posters situated in various locations around the University campuses and in community health centres in the local area. The advert was also posted on electronic forums and a social networking site.

During an initial pre-screen, BMI (previously self-reported) was measured. Height (without shoes) and weight were recorded to the nearest 0.1 cm and 0.1 kg respectively using SECA scales and stadiometer (SECA 709 mechanical column scales with SECA 220 telescopic measuring rod; SECA Birmingham, United Kingdom). Height measurements were made at the point of normal breath inspiration with the head positioned in the Frankfort horizontal plane. Percentage body fat and water were measured using bioelectrical impedance analysis (BodyStat 1500; BodyStat Ltd., Isle of Man, British Isles) while the participant was lying in the supine position on non-conducting foam matting in accordance with the manufacturer's guidelines. Participants were asked to complete the Three Factor Eating Questionnaire-R18 (TFEQ-R 18) (Karlsson, Persson, Sjostrom, & Sullivan, 2000), an adapted version of the 51-item TFEQ designed by Stunkard and Messick (1985). The TFEQ-R 18 is a self administered questionnaire used to assess eating restraint, uncontrolled eating and emotional eating. Its validity has been successfully evaluated in both obese (Karlsson et al., 2000) and normal weight populations (Hyland, Irvine, Thacker, Dann, & Dennis, 1989).

The intervention phase occurred over a period of 3 days. On day 1, participants were required to abstain from physical training activities and alcohol consumption and to fast overnight for 12 h (8 pm–8 am). At the beginning of day 1, participants started recording a 3 day estimated measures diet diary (guidance was given during the pre-screen session). This method of dietary assessment requires participants to estimate the amount of food consumed, using household measures, and record this in a standardised diary. Information recorded included method of cooking, brand names, as well as details of any food that was left over.

At 8:30 on day 2, anthropometric measurements (as described previously) were taken and baseline capillary blood samples were

	Control bread	Ascophyllum no	Ascophyllum nodosum enriched bread					
Ascophyllum nodosum	0	1	2	3	4			
Water	34	34	35	35	35			
Wholemeal flour	60	58	57	56	55			
Sugar	1	1	1	1	1			
Yeast	2	2	2	2	2			
Butter	3	3	3	3	3			

Table 1									
Ingredients	in t	he	control	and	enriched	bread	(displayed	as	%).

Percentages are rounded to 0 decimal places.

All loaves were baked from 450 g dough to produce a 400 g loaf.

collected from the finger tip using a single use Accu-chek® Softclix<sup>®</sup> Pro lancing device (Roche Diagnostics Ltd., West Sussex, UK). Thirty microliter of blood was collected in Microsafe Collection and Dispensing Tubes (Inverness Medical, Cheshire, UK), applied immediately to the sample area of a Reflotron<sup>®</sup> Cholesterol Test Strip and inserted into the Reflotron<sup>®</sup> dry chemistry analyser. Total blood glucose was measured using a single droplet of capillary blood applied to a OneTouch® Ultra® Test Strip with Fast-Draw<sup>™</sup> design which was inserted into a OneTouch<sup>®</sup> Ultra<sup>®</sup> Blood Glucose Monitoring System (reference range 1.1-33.3 mmol/L; Lifescan Inc., Bucks, UK). Participants were also asked to rate their baseline perceived hunger and fullness, along with 6 other 'distracter ratings' ("how friendly/nauseous/thirsty/happy/ energetic/relaxed do you feel?") on 100 mm visual analogue scales (VAS) with left end points anchored at "not at all" and right end points anchored at "extremely".

At 09:00 participants were asked to complete a second, identical VAS and consume a breakfast consisting of scrambled eggs on 100 g of either the *Ascophyluum nodosum* enriched bread (intervention arm; 4% *A. nodosum*/400 g loaf), or standard wholemeal bread (control arm: 0% *A. nodosum*) in the specialist feeding facility at the laboratory.

The feeding facility is temperature controlled (22–24 °C) with standardised 'natural' lighting, and positive-air flow. Participants followed a "silent" protocol in individual booths and were instructed to consume all the food provided. Bread samples were toasted for 1 min on each side and topped with scrambled eggs, (ingredients described by McCance and Widdowson in The Composition of Foods (Food Standards Agency & Institute of Food Research, 2002)). Scrambled eggs were cooked in a microwave for 1 min, stirred, and cooked for a further 30 s. It is estimated that the bread enriched with 4% A. nodosum contained 4.6 g alginate per loaf (1.15 g per serving), compared with 0 g in the seaweed-free control bread (Seagreens, 2006). It is important to note that these values are based on information received from Seagreens® and exact amounts were not tested. The energy content of the control bread and enriched bread was 214 kcal and 213.7 kcal respectively. Breads were coded to ensure blinding of conditions and participants were asked to rate the pleasantness of the meal using an electronic VAS on the Sussex Ingestion Pattern Monitor (SIPM version 2.08, University of Sussex).

Over the subsequent 4 h, an additional 10 capillary blood samples were taken (09:30, 09:45, 10:00, 10:15, 10:30, 10:45, 11:00, 11:15, 11:30, 12:00, 12:30, 13:00) and an equal number of paper-based VAS questionnaires were completed following each blood collection. Blood samples were tested for glucose and cholesterol using procedures described earlier. Participants could drink  $\leq 1$  l water *ad libitum* over the course of the morning. Water bottles were weighed prior to distribution and after 13:00 to quantify how much water had been consumed.

At 13:00 participants returned to the feeding facility to consume an *ad libitum* meal of Don Mario 100% durum wheat semolina penne pasta (Abbey Foods Ltd., Liverpool, UK) with Sacla Italia Vine–ripened Tomato & Mascarpone Stir Through sauce (Fratelli Sacla, S.p.A., Asti, Italy). This test meal was eaten in the feeding facility and Sussex Ingestion Pattern Monitor (SIPM) software was used to covertly weigh how much food was consumed. SIPM was developed from the Universal Eating Monitor and has subsequently been used in many appetite and sensory studies (Bertenshaw, Lluch, & Yeomans, 2008; Yeomans, Gould, Leitch, & Mobini, 2009; Yeomans, Weinberg, & James, 2005; Yeomans et al., 2008). Upon entering the feeding facility, participants completed the hunger and fullness VAS, along with a series of "mood ratings" which measured how they were feeling and to further distract them from the true purpose of the study. These ratings were the same as those measured on the paper-based VAS utilised earlier. Following this, participants were provided with the test meal and were instructed to "eat until you are comfortably satisfied" (Flint, Nikolaj, Gregersen, et al., 2007). At 100 g intervals, participants were asked to rate the pleasantness of the food, and then to continue eating. When each participant had consumed 300 g of their meal (i.e. reached the "refill weight") they were asked to call the experimenter who provided a new bowl of food, identical to the previous serving. At the end of the meal, participants were asked to confirm that they had finished eating, and were again asked to complete the hunger, fullness and mood ratings. Neither the order of in which rating scales were presented, nor the polarity of these scales were randomised however participants were not able to refer to previous ratings (Flint, Raben, Blundell, & Astrup, 2000), reducing carryover and/or memory effects. Following the consumption of lunch, participants left the feeding facility, continued with their usual routine, and continued to complete their food diaries.

During the follow-up stage (day 3), participants continued to record their estimated measures food diary in a free-living environment. They were contacted by a researcher and asked to recall what they had consumed during the 24 h immediately post intervention. The Automated Multiple Pass Method (AMPM) was used to collect food intake information over a 24 h period, not including food supplements (Raper, Perloff, Ingwersen, Steinfeldt, & Anand, 2004). These data were used to cross-check the food diaries for accuracy. NetWISP (version 3.0 for Windows, Tinuviel Software, Warrington, UK) was used to analyse all dietary data.

Data are presented as means  $\pm$  standard deviations and graphs were prepared in Microsoft Excel 2007. Blood measurements taken from 0–240 min allowed area under the curve (AUC) data to be produced using NCSS (Hintze, 2007. NSCC, PASS & GESS. NCSS. Kaysville, Utah). AUC data were also produced for hunger and fullness ratings over the same time period. Paired samples *t*-tests and Pearson correlation coefficients were carried out using SPSS version 17.0 (SPSS Inc., Chicago, USA). In all analyses, the accepted alpha level of significance was p < 0.05.

#### Results

## Study 1: Acceptability results

Seventy-nine untrained sensory panellists (40 males and 39 females) were recruited; all of whom successfully completed the

Table 2			
Sensory cha	racteristics of bread	containing Ascophyllum	nodosum

Amount of Ascophyllum nodosum per 400 g loaf (%)	0		1 1.15		2 2.3		3 3.45		4 4.6	
Estimated amount of alginate per 400 g loaf (g)										
	М	SD	М	SD	М	SD	М	SD	М	SD
Appearance	6.42 <sup>a</sup>	1.80	6.46 <sup>a</sup>	1.58	6.41 <sup>a</sup>	1.38	6.58 <sup>a</sup>	1.38	6.45 <sup>a</sup>	1.39
Aroma	6.38 <sup>a</sup>	1.55	6.14 <sup>a</sup>	1.45	6.06 <sup>a</sup>	1.53	6.30 <sup>a</sup>	1.55	6.09 <sup>a</sup>	1.44
Flavour <sup>*</sup>	6.31 <sup>b</sup>	1.83	5.56 <sup>a</sup>	1.74	5.50 <sup>a</sup>	1.74	5.67 <sup>a,b</sup>	1.65	5.52 <sup>a</sup>	1.75
Aftertaste <sup>¥</sup>	6.34 <sup>b</sup>	1.67	5.58 <sup>a</sup>	1.59	5.63 <sup>a</sup>	1.59	5.70 <sup>a</sup>	1.50	5.54 <sup>a</sup>	1.70
Texture	6.44 <sup>a</sup>	1.80	5.94 <sup>a</sup>	1.62	6.14 <sup>a</sup>	1.62	5.92 <sup>a</sup>	1.72	6.00 <sup>a</sup>	1.71
Overall acceptability§	6.60 <sup>b</sup>	1.68	5.79 <sup>a</sup>	1.52	5.95 <sup>a</sup>	1.52	5.93 <sup>a</sup>	1.59	5.86 <sup>a</sup>	1.64

Data are presented as means and standard deviations.

Different letters in the same row denote means that are significantly different to one another. Cut off for overall acceptability was 5 (Mexis et al., 2010).

\* *p* = .008.

p = .003.

§ p = .002.

acceptability tests. Importantly, all the breads were rated by panellists as acceptable overall and for each individual sensory attribute (Table 2). The control bread was rated significantly higher than the *A. nodosum* enriched bread for overall acceptability (p = 0.002) and for aftertaste (p = 0.003), and significantly higher than all but the bread enriched with 3% *A. nodosum* for flavour (p = 0.008). *Post hoc* tests showed no significant differences between any of the enriched breads. Interestingly, the bread containing 4% *A. nodosum* was considered slightly more acceptable overall (5.86) than the product containing 1% *A. nodosum* (5.79) although this was not significant.

As a result of these findings, the bread containing 4% *A. nodosum* per 400 g loaf was selected for use in the breakfast meal of the satiety study.

## Study 2: Satiety results

Twelve males were recruited for the satiety study (age  $40.1 \pm 12.5$  years; BMI  $30.8 \pm 4.4$  kg/m<sup>2</sup>). One participant was excluded from the dietary analysis due to incomplete diet diaries, and a different participant was excluded from cholesterol analyses as fasted blood results indicated hypercholesterolaemia (>6.5 mmol/L, Engbers, van Poppel, & van Mechelan, 2007; Musial et al., 2001).

Energy intake at the test meal following the ingestion of *A. nodosum* enriched bread was 747.7 kJ (178.7 kcal), 16.4% lower (p = 0.006) than energy intake following the consumption of the

control bread (mean =  $3825.0 \pm 1590.6$  kJ ( $914.2 \pm 380.2$  kcal) and  $4572.7 \pm 1927.5$  kJ ( $1092.9 \pm 460.7$  kcal), respectively).

During the 24 h, free living period after participants left the feeding facility (referred to in Fig. 1 as "post test meal"), energy intake was lower in the intervention arm of the trial compared to the control arm (8974.7 ± 3365.2 kJ (2145.0 ± 804.3 kcal) and 10303.1 ± 2356.8 kJ (2462.5 ± 563.3 kcal), respectively) although this difference of 1326.3 kJ (317 kcal) was not significant (p = 0.133).

Total energy intake (test meal energy intake + post test meal energy intake) was significantly lower (2117.5 kJ (506.1 kcal); p = 0.007) following the consumption of the enriched bread (12914.3 ± 4428.3 kJ (3086.6 ± 1058.4 kcal)) compared to the control bread (16538.1 ± 3307.5 kJ (3592.7 ± 790.5 kcal)).

Differences between treatment arms for AUC, peak values, and time of peak for blood glucose and cholesterol and for hunger and fullness were not significant, although the time at which post-prandial peak hunger was reached was considerably delayed following consumption of the *A. nodosum* bread compared to the control bread reached (191.3 ± 94.2 min vs.  $115.0 \pm 120.6$  min; p = 0.055).

The pleasantness of the *A. nodosum* enriched bread was not significantly different to the control bread when consumed as part of the breakfast meal in the satiety study, suggesting participants were successfully blinded to each treatment. There was no significant difference in the amount of water consumed between breakfast and the test meal on either arm of the trial.



**Fig. 1.** Energy intake at various time points during and post-intervention. A = *Ascophyllum nodosum* enriched bread; C = control bread; test meal = *ad libitum* lunchtime feed; post test meal = 24 h energy intake after test meal (free living environment); total = test meal + post test meal.

#### A. nodosum enriched bread is acceptable

Results from the acceptability tests show that *A. nodosum* can be successfully incorporated into a 400 g wholemeal loaf at concentrations of up to 4% whilst maintaining acceptability. This is encouraging for the food industry, particularly the bakery sector who may wish to incorporate seaweed such as *A. nodosum* not only as a potentially satiating ingredient, but also as a salt replacer and antimicrobial agent (Gupta & Abu-Ghannam, 2011). Importantly, the breakfast meal used in the satiety study was independently deemed to be acceptable in a suitable preliminary experiment (study 1). Although participants were in effect reporting the acceptability of scrambled eggs on toast, any differences in scores between samples are attributable to the bread rather than other components.

An analysis of the bread using a combination of the SIGMA and Fibertech methods showed the bread enriched with A. nodosum at concentrations of 4% (17.8/100 g) contained 4.5 g more dietary fibre/100 g than the control bread (13.3/100 g). Thus all samples were classified as high fibre foods. Traditionally, high fibre foods tend to be solid (Slavin & Green, 2007) and have low level palatability, making them less organoleptically appealing than high energy dense alternatives (Burton-Freeman, 2000). However Gomez, Ronda, Blanco, Caballero & Apesteguía. (2003) suggest two reasons for adding dietary fibre to bakery products: firstly, to increase the overall fibre content of the product, and secondly, to decrease the energy density. Dietary fibres have been successfully added to a wide variety of food matrices including bakery products, cereals, pasta noodles and a variety of beverages (Brennan & Cleary, 2007; Collar, 2003; Collar, Santos, & Rosell, 2006; Collar, Santos, & Rosell, 2007; Hall, Baxter, Fryirs, & Johnson, 2010; Rosell, Santos, & Collar, 2006; Santos, Rosell, & Collar, 2008). The addition of lupin kernel fibre to white bread and pasta resulted in no significant differences in overall acceptability (n = 44) (Clarke & Johnson, 2002), neither did the addition of carob fibre, inulin or pea fibre to bread (Wang, Rosell, & Benedito, 2002). Similarly, Gomez and colleagues (2003) found the addition of 2% orange, pea or wheat fibre to flour enhanced textural shelf life and showed no deterioration in palatability. Indeed, Angioloni and Collar (2011) found an increase in overall acceptability after the addition of a binary mixture of cellulose and either fructo-oligosaccharide or gluco-oligosaccharide. This, coupled with maintaining shelf life for 10 days, suggests that dietary fibre can be successfully added to bread from both a physical and sensorial perspective.

It is easier to maintain the acceptability of fibre enriched foods when fibrous isolates are added to products rather than wholefood ingredients. Previous studies have incorporated sodium alginate into beverages (Hoad et al., 2004; Paxman et al., 2008a,b; Wolf et al., 2002) and a few have developed food products such as crispy bars (Williams et al., 2004) and breakfast bars (Mattes, 2007). The amounts of alginate used in these studies (1.6 and 1.1 g, respectively) are comparable to the approximate amount of alginate believed to be found in the bread containing A. nodosum. Most authors (Hoad et al., 2004; Paxman et al., 2008a, 2008b; Williams et al., 2004; Wolf et al., 2002), but not all (Mattes, 2007) have reported beneficial health effects at these levels. Alginate (and separately, other hydrocolloids such as carageenan, xanthan and hydroxypropylmethylcellulose (HPMC)) have also been added to bread (0.1% and 0.5%), showing a reduced loss of moisture and dehydration rate due to their ability to retain water. A trained sensory panel (n = 10) scored all samples as acceptable, with the highest scores from the alginate (0.5%) and HPMC enriched (0.1%) samples (Guarda, Rosell, Benedito, & Galotto, 2004).

Whilst the addition of marine extracts (such as alginate) to bread and bakery products has been successful, to date, the effect of adding whole seaweed to bread has not been widely investigated. Prabhasankar, Ganesan, and Bhaskar (2009) added brown seaweed (*Sargassum marginatum*) to pasta, enhancing biofunctional characteristics, and Prabhasankar, Ganesan, Bhaskar, et al. (2009) showed that the addition of *Undaria pinnatifida* to bread (up to 10%) was sensorialy acceptable with no significant differences between the control (0%) and 5% breads, or between the 5% and 10% breads. Acceptability was significantly reduced at levels greater than 10%. No studies investigating the potentially satiating effects of whole seaweed in bread have been published to date. The successful incorporation of whole seaweed (*A. nodosum*) into bread meant that the bread containing the highest amount of seaweed 4% could be used in the subsequent satiety study.

# A. nodosum enriched bread decreases energy intake at a test meal

This study has shown for the first time that A. nodosum enriched bread, consumed within a composite breakfast meal of scrambled eggs on toast, can significantly lower energy intake at a meal served 4 h later in overweight but otherwise healthy males. Mean energy intake was significantly lower (747.7 kJ (178.7 kcal); p = 0.006) following the consumption of the A. nodosum enriched bread compared to the control bread. Other laboratory based studies have found a reduction in energy intake following the consumption of lupin fibre enriched bread (Lee et al., 2006) and an alginate-pectin combination fibre (Pelkman, Navia, Miller, & Phle, 2007) reduced energy intake by approximately 10% (p = 0.11). However, similar to the current study, these were acute, laboratory based feeding studies which do not emulate free living situations well. The current study was small and well controlled, with high internal, yet low external validity. While laboratory based studies such as this enable rigorous control and considerable precision with little influence from external factors, they are too short to make definitive statements about long term energy balance (Stubbs, Johnstone, O'Reilly, & Poppitt, 1998). These acute feeding studies are suitable precursors to longer term, free living experiments although there appear to be relatively few examining the relationship between fibre and energy intake. Paxman et al. (2008a) report a daily energy deficit of 135 kcal in adults (n = 68) while consuming an alginate based beverage (1.5 g alginate) for 7 days. Similarly, Cani, Joly, Horsmans, and Delzenne (2006) report a daily energy intake reduction of 120 kcal with the consumption of 8 g oligofructose a day in a small pilot study, and Pasman, Saris, Wauters, and Westerterp-Plantenga (1997) fed large amounts of guar gum (40 g/day) to 17 participants, reporting a substantial daily energy deficit of 310 kcal/ day. No previous acute laboratory based studies, or long term, free living studies have examined the relationship between whole seaweed and appetite. A free living study is warranted; a daily energy deficit of ~100 kcal may help prevent weight gain (Hill, Wyatt, Reed, & Peters, 2003; Lean, Lara, & Hill, 2006), and whilst we have shown this to be eminently achievable in a laboratory setting, the application of these findings to the general, free-living population is limited.

Total energy intake (energy intake from test meal combined with 24 h energy intake) was significantly lower (2117.5 kJ (506.1 kcal); p = 0.007) following the treatment compared to the control bread. A habitual energy reduction of ~500 kcal/day may be beneficial in long term sustained weight loss (Astrup, 1999) which may reduce the risk of type 2 diabetes mellitus (Moore, Visioni, Wilson, et al., 2000) and hypertension in overweight and obese individuals (Moore et al., 2005).

#### A. nodosum enriched bread has no effect on nutrient uptake

There were no significant differences in AUC glucose or cholesterol following the consumption of the *A. nodosum* enriched bread compared to the control. Peak glucose values of 6.9 mmol/l were reached at 75 min for both treatments and there were no significant differences in cholesterol levels at any time point throughout the intervention. In a small (n = 14) yet well controlled pilot study, Paxman et al. (2008b) showed that compared to a control (containing no alginate), the consumption of a beverage containing 1.5 g sodium alginate significantly ameliorated the increased glucose and cholesterol uptake found in individuals with a higher body fat percentages compared to those with lower body fat percentages. Wolf et al. (2002)(n = 30) also added alginate (3.6 g) to a beverage and while no difference was seen in peak glucose, a significant (p < 0.01) decrease in AUC glucose was apparent when compared to the control. Torsdottir, Alpsten, Holm, Sandberg, and Tã-lli, (1991) saw a reduced rise in glucose (p < 0.02) and a slower rate of gastric emptying (p < 0.05) in 7 diabetic males following the consumption of 5 g sodium alginate compared to a control. Each of these studies used sodium alginate, a seaweed isolate, and suggested that the modulated glycaemic response was due to gelation of alginate causing a slower rate of gastric emptying and possible nutrient encapsulation. One study used whole red seaweed (Nori) in a capsule form (3 g) and measured the post-prandial glucose response to white bread consumed 15 min later. The authors concluded that Nori seaweed significantly ( $p \leq 0.05$ ) reduced AUC glucose, and again, postulate that delayed gastric emptying was the mechanism of action (Goni, Valdivieso, & Garcia, 2000). Previous studies have described how the inclusion of dietary fibre, may reduce blood cholesterol levels, and various mechanisms have been described (Behall, Scholfield, & Hallfrisch, 2004; Braaten et al., 1994; Brown, Rossner, Willet, & Sacks, 1999; Gunness, Flanagan, & Gidley, 2008; Jeminez-Escrig & Sanchez-Muniz, 2000; Ripsin, Keenan, & Jacobs, 1992; Sola et al., 2010; van Horn et al., 1991). These benefits were not apparent in the present study, however this may be attributable to the small sample size and therefore limited statistical power. Nutrient uptake in the current study does not appear to be slowed or reduced, suggesting neither gelation nor nutrient encapsulation occurred. A more likely mechanism here is that the seaweed acted as a bulking agent, increasing gastric stretch to a greater extent than standard wholemeal bread. It is also possible that an altered gut peptide response mediated enhanced satiety or brought about premature satiation at the subsequent test meal. The mechanism(s) of action for the observed effects warrant further investigation, particularly in studies using larger cohorts. As the energy contents of the bread were very similar (control 214 kcal/100 g; enriched bread 213.7 kcal/100 g) it is highly unlikely that this would have influenced energy intake at the test meal.

The discordance between the nutrient uptake findings from the present study and others in the published literature base may be explained by the small amount of alginate present in the *A. nodosum* enriched bread consumed (participants in the present study consumed 100 g of bread, containing an estimated 1.15 g of alginate). This amount is not dissimilar to that used by Mattes (2007) who incorporated 1.1 g sodium alginate into a breakfast bar and suggested that the lack of effect of the product on appetite ratings and energy intake over 5 days was due to the low amounts of alginate used, which lead to poor gelation in the stomach. It is also possible that in this study, alginate was entrapped within the seaweed particles. Amounts of alginate in the current study are estimates based on the nutritional profile of *A. nodosum*, and it is unlikely that intra-gastric gelation occurred.

# A. nodosum enriched bread does not alter hunger and fullness ratings

There were no significant differences for total AUC hunger or fullness at any time point throughout the intervention between the *A. nodosum* enriched bread and control bread. Interestingly

though, peak hunger was reached over 1 h later (76 min) following the consumption of the enriched bread vs. the control bread, however this did not reach significance (p = 0.055). This delay in peak hunger could have potentially contributed to the reduced amount of energy consumed at the test meal.

# Compliance: Study 2 (satiety study)

While the sample size was small, the study was well controlled. Compliance to the protocol was high; one participant consumed a small amount of alcohol (5.3% of total energy intake) at lunchtime on day 1, and a different participant took part in training type activities on the morning of day 1. It is unlikely that these activities had an effect on the overall outcome of the study. As instructed, all participants consumed the breakfast provided in its entirety. Participants were blind to the treatment they received, and did not report any significant differences in the pleasantness, or other flavour attributes of the bread suggesting that they were unaware of which treatment they received. From the debrief session it became apparent that participants were unaware of the weighing scales concealed within the feeding facility, ensuring the food consumed during the test meal was covertly weighed.

# Conclusion

This study has shown for the first time that the incorporation of *A. nodosum* into bread significantly reduces energy intake at a subsequent test meal. The total energy intake in the 24 h period following consumption of the *A. nodosum* enriched bread was also significantly reduced although energy intake during the free-living (post test meal) period was not altered. No significant differences were seen in AUC glycaemia or cholesterolaemia which suggests that neither delayed gastric emptying nor nutrient encapsulation occurred. There were also no significant differences in AUC hunger or fullness. Further investigation of potential mechanisms of action is warranted.

This study was an acute feeding trial. Incorporating *A. nodosum* into a long term, appropriately powered, free living intervention study involving the substitution of "normal" bread for *A. nodosum* enriched bread, would help to establish the potential for seaweed enriched bread to reduce habitual energy intake longitudinally, with potential to favourably affect BMI or body composition.

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